THE CAMBIUM AND ITS DERIVATIVE TISSUES NO. X. STRUCTURE, OPTICAL PROPERTIES AND CHEMICAL COMPOSITION OF THE SO-CALLED MIDDLE LAMELLA

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INTRODUCTION

MUCH HAS BEEN WRITTEN during the last hundred years concerning the structure, the development, and the physical and chemical properties of the cell wall. A careful study of this literature reveals many varied and more or less contradictory points of view. For example, there is no consensus of opinion concerning the use of such terms as *intercellular substance, middle lamella, primary wall, secondary wall, tertiary wall,* etc. Each of them is employed in several fundamentally different senses and to designate entirely different structures. It is essential to clarify this situation, since the existing confusion results in serious discrepancies, not only in descriptive morphological work, but also in physiological, biophysical, and biochemical investigations.

WHAT CONSTITUTES THE SO-CALLED MIDDLE LAMELLA?

Von Mohl (18) and the earlier botanical investigators believed that the cells of the xylem and of other tissues are bound together by a refractive intercellular substance which differs from the cell walls in its solubilities and other properties. Subsequently, with the discovery of cell division and an altered viewpoint regarding the origin and development of cells, this refractive layer was referred to by Hofmeister (14), Sachs (22), and many other botanists, as the middle lamella. The cytological investigations of Treub (32), Strasburger (30), Timberlake (31), and Allen (1) ultimately led to the conclusion that the middle lamella originates between the halves of the split cell-plate and is, in fact, the first-formed membrane or partition wall, more or less modified in thickness and in chemical composition during tissue differentiation.

Most botanists have differentiated the middle lamella by its refractive character, its differential staining, or its solubility in macerating agents. Dippel (9) demonstrated, however, in the first edition of "Das Mikroskop" that the refractive layer, which appears more or less homogeneous in white light, consists of three distinct layers, i. e., two anisotropic layers and an intervening isotropic layer, *Fig. 5*. He designated the anisotropic layers as primary walls and the isotropic central layer as intercellular substance, the three layers combined constituting the middle lamella. More recently there has been a tendency, among certain investigators, to limit the term middle lamella to the putative isotropic layer. Thus, Ritter (19) has defined the middle lamella as "the isotropic peripheral layer of the cell wall, including the irregular masses of isotropic material commonly found where three or more cells adjoin." Van Iterson (33) similarly differentiates the central isotropic layer as the original or "true middle lamella" and refers to Dippel's middle lamella as the "compound middle lamella."

Mangin (15, 16) demonstrated that the middle lamella of soft tissues is composed of pectic substances, and that "pectose" is intimately associated with cellulose in cell walls which have not been modified by lignification, suberization, etc. He inferred, from macerations of meristematic tissues, that the original partition membrane is of pectic composition and that cellulose is first deposited during the process of secondary thickening. In recent years, Ritter (19), Harlow (12), and others have emphasized the fact that a large proportion of the lignin in wood is located in the middle lamella. Furthermore, Schorger (28) and Ritter (20) consider that the middle lamella is actually composed of lignin.[1]

In view of the existing confusion concerning what constitutes the so-called middle lamella and regarding its physical and chemical constitution, it seemed desirable to the writers to undertake an extensive investigation of various tissues in an endeavor to clarify the situation. In order to have a clear conception of the so-called middle lamella in the mature xylem, it is necessary to consider the conditions in the cambium and the changes which take place during tissue differentiation. The results of a detailed study of the cambium and of its differentiating and fully differentiated derivatives are recorded in the following pages.

[1]It should be noted in this connection, in view of various criticisms of botanical workers, that not only did Schacht (24), Strasburger (29), Dippel (10), and many other botanists subject sections of tissues to chemical treatments, but also that they were cognizant of the fact that the middle lamella of woody tissues is insoluble in concentrated sulphuric acid and in Schweizer's reagent.

MATERIAL AND METHODS

In our work on the cambium, the following species were studied:

1. Pinus Strobus L.
2. Juniperus virginiana L.
3. Thuja occidentalis L.
4. Robinia Pseudoacacia L.
5. Cladrastis lutea (Michx. f.) Koch
6. Fraxinus americana L.
7. Acer rubrum L.
8. Catalpa speciosa Warder
9. Liquidambar Styraciflua L.

Most of the original observations were made on *Pinus Strobus*, but every phase of the work was checked and rechecked on other species. In dealing with the cambium, it is essential to secure freshly collected material from living trees. This is due to the fact that cambial walls contract so much during dehydration that permanent preparations present a caricature of the original structure.

Transverse and longitudinal sections of the wood of more than 800 genera of gymnosperms and angiosperms were examined in polarized light. The following species were selected for subsequent microchemical investigation.

1. Pinus Strobus L.
2. P. radiata D. Don
3. Taxodium distichum (L.) Richard
4. Sequoia sempervirens Endl.
5. Libocedrus decurrens Torr.
6. Trochodendron aralioides Sieb. & Zucc.
7. Ulmus americana L.
8. Tilia glabra Vent.
9. Quercus agrifolia Née
10. Q. Douglasii Hook. & Arn.
11. Prosopis juliflora DC.
12. Citrus Limonia Osbeck
13. Betula papyrifera Marsh.
14. Populus grandidentata Michx.
15. P. trichocarpa Torr. & Gray
16. Nyssa sylvatica Marsh.
17. Robinia Pseudoacacia L.
18. Liquidambar Styraciflua L.
19. Catalpa speciosa Warder
20. Fraxinus mandshurica Rupr.

21. Rhizophora mangle L.
22. Myodocarpus simplicifolius Brong. & Gris
23. Protomegabaria Stapfiana Hutch.
24. Homalium guianense (Aubl.) Warb.
25. Brackenridgea Hookeri A. Gray
26. Tetramerista glabra Miq.

The last six species are tropical forms which were used for comparison with the species of warm-temperate and cold-temperate regions. Sections were made either from freshly collected wood or from seasoned wood soaked in cold water. No softening agents were employed, as these change the chemical properties of the cell walls.

In studying the optical properties of wood, thin and truly transverse sections of straight-grained specimens are essential. Most of the critical work with polarized light was done on sections 5 μ in thickness. Thicker or slightly oblique sections obscure the finer details and give much distorted images. Chemical tests were made on sections 5 to 15 μ in thickness, depending upon the ease with which the material could be cut.

THE CAMBIUM

Since 1900, most botanists have visualized the cambium as an extremely delicate and very thin-walled tissue. Such a conception is due largely to the study of fixed and dehydrated material and to unfamiliarity with the investigations of Sanio (23), Schacht (25), Strasburger (29), Dippel (10), and others who worked with freshly cut and undehydrated material. Sanio (23) demonstrated that the radial walls of the fusiform initials in the cambium of *Pinus sylvestris* are of considerable thickness and that those of adjacent cells are separated by an amorphous intercellular layer or "Zwischensubstanz." Sanio (23) and others erred, however, in their assumption that the occurrence of a "Zwischensubstanz" is an unusual phenomenon in meristems and involves the splitting of an originally entire and homogeneous partition membrane. If they had devoted more attention to the study of ray initials and to the cambia of dicotyledons, they might have recognized the fact that each initial is enclosed, *on all sides,* within a wall of its own which is separated from the walls of adjoining cells by more or less intercellular material. The cambial wall expands as the initial increases in size. The intercellular material is a plastic colloidal substance that passes readily into a semi-liquid phase, thus facilitating those movements and adjustments of cells which are such characteristic features of the actively growing cambium.[1]

[1]For a discussion of these and other phenomena and a description of techniques employed in studying the living cambium, the reader is referred to preceding papers of this series, Bailey (2, 3, 4), and Bailey and Zirkle (5).

In other words, the wall of the cambial initials is a discrete morphological structure which, once formed, maintains its identity under all conditions of growth and development, whereas the intercellular layer is passively molded into various forms and possesses few of the attributes of a true membrane.

Dippel (10) concluded, in the second edition of "Das Mikroskop," that the walls of the cambium are isotropic. He figured (Part II, page 573) a transverse section of the cambium and its derivatives as seen in polarized light with crossed nicols. The walls of the initials and of the enlarging derivatives are dark, whereas the "primary walls," formed during the later stages of the differentiation of the xylem and phloem, are brilliant. This was in line with Dippel's contention that the cambial walls—which are incorporated in the isotropic "intercellular substance" of mature tissues—are composed of "pectose," whereas the subsequently formed "primary walls" consist largely of cellulose.

In view of the accuracy of Dippel's delineations of polarized light pictures of mature tissues, which are much superior to those of recent workers, it is difficult to understand how he could have been led so far astray in his treatment of the cambium. *Figure 1* is a transverse section of the actively growing cambium of *Pinus Strobus,* photographed in polarized light with crossed nicols. Although there is a more intense anisotropy in the region of the xylem than in the cambial zone, the walls of the meristematic cells and of their enlarging derivatives obviously exhibit double refraction. *Figure 2* is a tangential longitudinal section of the cambium of *Fraxinus americana,* likewise photographed in polarized light with crossed nicols. It is evident that the walls of both ray initials and fusiform initials are birefringent. Furthermore, an examination of transverse and longitudinal sections of the cambia of a wide range of gymnosperms and angiosperms indicates that all cambial walls are anisotropic, but that the intensity of double refraction, angles of extinction, etc. vary somewhat from plant to plant. The anisotropy is not due to "rod" or "plate" double refraction, since the walls are birefringent even when they are saturated with a liquid of the same refractive index as their own (Frey, 11). The intercellular substance, on the contrary, is truly isotropic and is dark at all angles and in all planes of section. *Figure 3,* a highly magnified portion of a thin tangential, longitudinal section of the cambium of *Pinus Strobus,* shows the anisotropic walls of two adjacent fusiform initials and the dark isotropic intercellular material between them.

It should be noted, in this connection, that cambial walls are characterized by having more or less numerous plasmodesmata which may be

uniformly distributed or aggregated in thinner areas of the walls, i. e., in so-called primary pit-fields. These primary pit-fields are more closely approximated in the radial walls of the fusiform initials of most dicotyledons, *Figure 4*, than they are in the case of gymnosperms, *Figure 3*. Thus, the walls have a beaded appearance in sectional view, *Figures 2, 4*, and *11*. The intercellular material is much reduced in amount in the region of the primary pit-fields and between the tangential walls of the fusiform initials of gymnosperms, but its presence may be clearly demonstrated by adequate modern techniques.

Our investigations of the cambium indicate that the isotropic intercellular material is composed largely of polyuronides and contains little or no cellulose, and that the anisotropic cambial walls contain both cellulose and polyuronides. When freshly cut sections of the living cambium are subjected to standard chemical treatments used in the extraction of pectic compounds, e.g., ammonium oxalate or dilute acids followed by dilute alkalis, the intercellular material dissolves, leaving the dissociated cambial walls, which are anisotropic and give a typical blue color with chloro-zinc iodide or iodine and sulphuric acid. The dissociated cambial walls are insoluble in 30% sodium hydroxide, or after prolonged treatments in hot dilute acids followed by alkalis, but dissolve completely in such standard solvents of cellulose as 72% sulphuric acid or Schweizer's reagent. When freshly cut sections of the cambium are used, the walls are also completely soluble in 72% sulphuric acid which dissolves both cellulose and polyuronides, but when treated with Schweizer's reagent, there remains a swollen residue of the cambial walls which is isotropic and no longer produces a blue color with iodine and sulphuric acid. This residue stains intensely with ruthenium red[1] and disappears in standard solvents for pectic compounds.

The physical properties, chemical solubilities, and colorations with ruthenium red, chloro-zinc iodide, and iodine-sulphuric acid indicate, therefore, that cambial walls are composed of a mixture of cellulose and polyuronides, and that they are separated by intercellular material which consists largely, if not entirely, of polyuronides. In other words, our observations and analyses are in substantial agreement with those of Mangin (16), Carré and Horne (7), and many others who have dealt with other types of soft, unlignified tissues. Furthermore, we have worked, during the last two years, in close coöperation with Professor Ernest Anderson, who has extracted the polyuronides from the cambium and young phloem of *Robinia Pseudoacacia* and has determined that they are of a pectic nature.

[1]For a discussion of the significance of the ruthenium red reaction see page 340.

The walls of cambial initials are characterized, from a physiological point of view, by their capacity for growth and expansion and for undergoing various reversible changes, e.g., seasonal variations in thickness and in colloidal properties. During the resting season, the cambial walls tend to be thicker and to have the general properties of a firm gel, whereas during the active growing season, they appear to be thinner and more pliable.

TISSUE DIFFERENTIATION

The specific changes that the cambial walls undergo during the differentiation of the xylem and phloem vary markedly, depending upon the particular types of tissue cells that are to be formed. In the case of those parenchymatous elements of the phloem, which retain their capacity for growth and enlargement, the original cambial walls are but slightly modified in form and thickness during tissue differentiation, and no true secondary walls are formed (compare *Figures 10* and *11*).

On the contrary, in the case of tissue cells which undergo irreversible changes and form layers of true secondary wall thickening, the cambial walls and the intercellular material tend to become thinner and considerably modified in form during the process of cell enlargement. Vestiges of the original primary pit-fields and intervening thickenings may persist, however, in parts of cells which are not subjected to excessive expansion. Such vestiges are of common occurrence in the radial walls of the tracheids of most conifers and of the terminal wood parenchyma of many dicotyledons, *Figures 12* and *13*.

The enlargement of those derivatives of the cambium which form tracheids, fiber-tracheids, libriform fibers, and bast fibers involves an increase in the cross-sectional area of the cells and elongation due to apical sliding growth. The cambial wall expands as the cell increases in size, but layers of secondary thickening are not deposited until the cell has attained its mature diameter. As the cambial walls increase in surface area and decrease in thickness, the layer of intercellular material becomes so tenuous that it is difficult to differentiate it by the use of ordinary microscopical methods, except in the angular spaces where three or more cells adjoin. Lignification is initiated during the earlier stages of secondary wall formation, and, in the species studied by us, is first visible in the cambial walls and the intercellular substance. It spreads centripetally through the successively formed layers of the secondary wall. The lignification of the cambial walls is usually very intense, except in the pit-membranes and crassulæ; whereas that of the secondary layers is extremely variable, particularly in dicotyledons.

The difficulty of differentiating the cambial walls from the intercellular material is accentuated by lignification, which alters the staining reactions and the indexes of refraction of the three adjoining layers, and makes them appear as a single homogeneously refractive unit. Furthermore, although the cambial walls are clearly anisotropic during the period of cell enlargement, *Figure 1,* their anisotropy tends to be obscured after the initiation of secondary wall formation and of lignification.

During the transformation of sapwood into heartwood the walls may absorb more or less "Gerbstoffe" which further alters their solubilities, optical properties, and staining reactions. Similarly, during the seasoning of sapwood, the walls may become saturated with various substances that are contained in the vacuoles of the living cells or are produced by the dying protoplasts. Therefore, critical observations should be made, if possible, upon freshly cut sections of living sapwood.

THE MATURE SECONDARY XYLEM

As will be shown in a subsequent paper of this series, the secondary wall is exceedingly complex, from a morphological point of view. It varies greatly not only in different tissue cells, but also in homologous walls of different plants.

In the case of normal tracheids,[1] *Text figure 1,* the secondary wall consists of three layers:[2] (1) a relatively narrow outer layer (c), (2) a narrower inner layer (e), and (3) an intervening layer (d) of variable thickness. The narrow inner and outer layers are characterized by having their fibrils of cellulose oriented more nearly at right angles to the longer axis of the cell, whereas the intervening layer is characterized by having them arranged either longitudinally or diagonally. The secondary walls project inward and are not in contact with the cambial walls in those parts of the cell where bordered pits are formed, *Text figure 2.* The narrow inner and outer layers of the secondary wall come together in the rim formed about the pit-aperture.

When unstained sections of wood are examined in white light, *Figure 8,* the narrow outer layers of the secondary walls of adjoining tracheids tend to blend with the cambial walls and the intercellular substance, forming an apparently homogeneous refractive layer, i. e., the so-called middle lamella of Dippel and of other botanical writers. That this middle lamella is actually a compound structure may be determined by

[1]Excluding "Rotholz" tracheids and other specialized and peculiar types.
[2]We are not concerned here with the submicroscopic layering which is revealed by swelling the walls and by other drastic treatments.

a careful study of the layering in the bordered pit-pairs (compare *Figure 8* and *Text figure 2*) or by transferring the sections to liquids of varying indexes of refraction.

When unstained transverse sections of wood are examined in polarized light with crossed nicols, the images vary considerably, depending

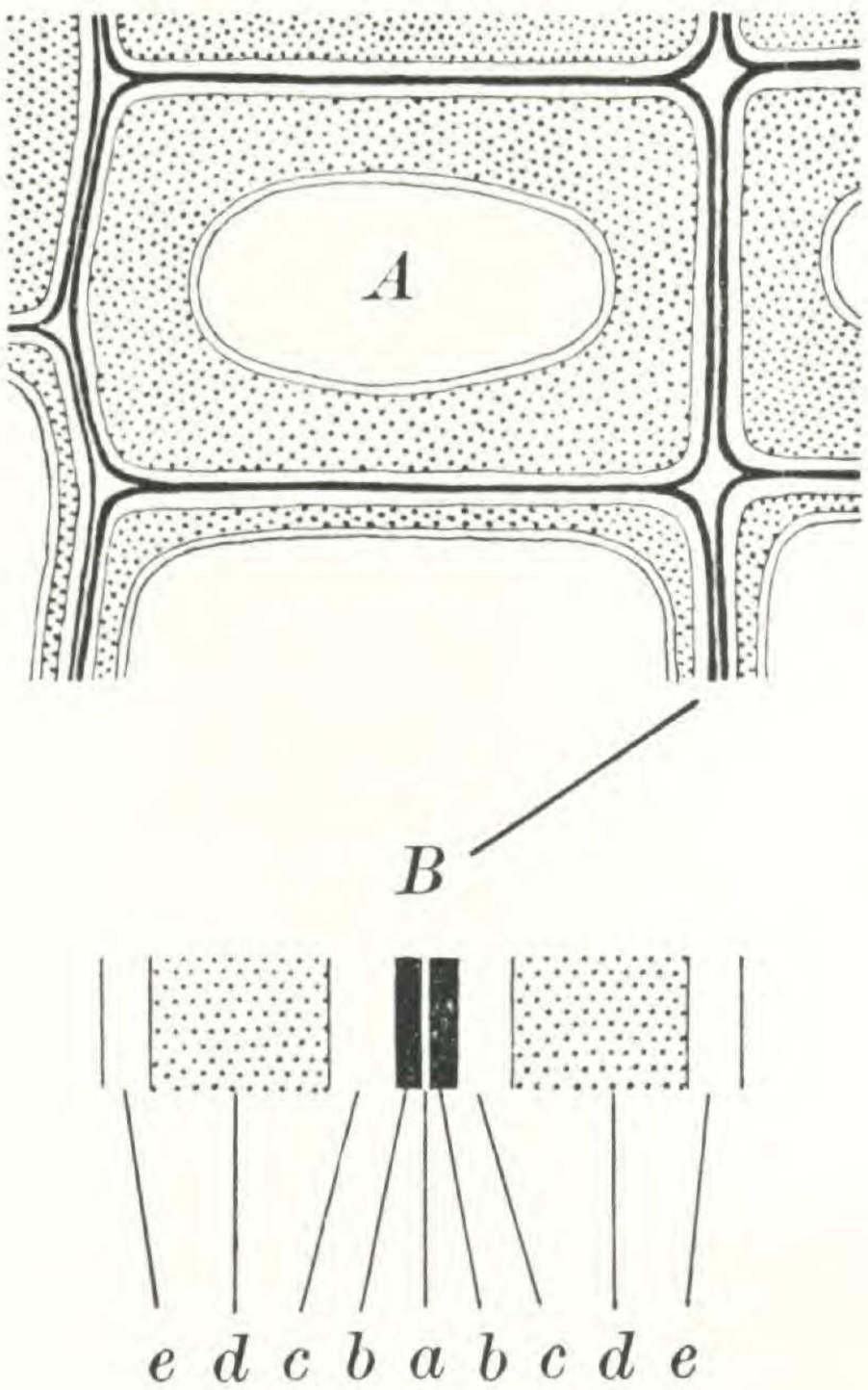

Figure 1

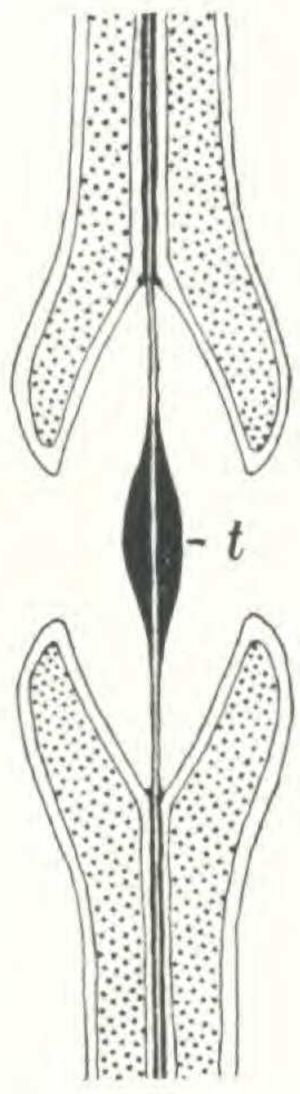

Figure 2

TEXT FIGURE 1. (A) Diagrammatic transverse section of one entire tracheid and of parts of seven others. (B) Section of adjacent walls more highly magnified; (a) truly isotropic intercellular substance, (b) cambial or primary wall, (c) outer layer of secondary wall, (d) central layer of secondary wall, (e) inner layer of secondary wall.

TEXT FIGURE 2. Diagrammatic bordered pit-pair in sectional view, showing pit-membrane, torus, and pit-apertures or openings in the secondary walls. Wall layers as in Text Fig. 1. t = torus or central thicker portion of the pit-membrane. The torus and the pit-membrane are composed of two cambial walls and of intercellular material.

upon a number of fluctuating factors. The narrow inner and outer layers of the secondary wall exhibit double refraction and are brilliant, *Figure 9*, whereas the central layer of the secondary wall is dark or less birefringent.[1] In other words, the phenomena observed between crossed

[1]The conditions tend to be reversed in longitudinal sections.

nicols are closely correlated with the orientation of cellulose micellæ. Where these are arranged parallel to the long axis of a tracheid, a layer appears dark in cross sections; where they are oriented nearly at right angles, a layer is brilliant. The brightness varies at intervening angles.

The closely approximated outer layers of the secondary walls of adjoining cells allow so much light to get by the crossed nicols that they fog or obscure, *Figure 9,* the three tenuous intervening layers, except in extremely thin sections, *Figures 5* and *6*. But in the case of the latter sections, the cambial walls and the intercellular material appear as a single isotropic layer, i. e., Dippel's intercellular substance, the so-called middle lamella of Van Iterson, Ritter, and others. The isotropic aspect of the cambial walls is due largely to the fact that they are so feebly anisotropic in comparison with the brilliant outer layers of the adjoining secondary walls, that they appear dark in contrast to them. Therefore, it is difficult to demonstrate the anisotropy of cambial walls when they are in contact with secondary walls, except under favorable circumstances. In the case of the mature xylem, the best material for this purpose occurs in those cells or portions of cells in which the cambial walls do not thin down excessively during tissue differentiation. Thus, in *Figure 7,* the feebly anisotropic cambial walls are visible between the brilliant outer layers of the secondary walls. By treating thin sections of the mature xylem with 65% phosphoric acid or diluted Schweizer's reagent, there is sufficient swelling so that it is possible to see the cambial walls as an anisotropic entity distinct from the outer layers of the secondary walls.

When woods are prepared for sectioning by the usual procedure of boiling in water and softening in hydrofluoric acid, the staining of sections with ruthenium red or with Haidenhain's haematoxylin produces the general appearance illustrated in *Figure 19*. The cambial walls and the intercellular material react as a unit—i. e., the so-called middle lamella or primary wall of various botanical writers—and stain more intensely in ruthenium red and haematoxylin than do the three layers of the secondary wall which react as another unit. It is possible to differentiate this homogeneously-staining so-called middle lamella into its constituent layers, not only by optical methods but also by chemical methods and differential staining. Thus, when sections of freshly cut wood, or of seasoned wood soaked in cold water, are treated with solvents for removing polyuronides, the cambial walls may be stained without appreciably coloring either the secondary walls or the intercellular material, *Figure 20*. By accurately controlled delignification, followed by treatment in Schweizer's reagent, it is possible to dissolve both the inter-

cellular material and the secondary walls, leaving the cambial walls intact, *Figure 15*. Owing to the fact that they may be unlignified, such minute and delicate structures as tori and pit-membranes may be resolved into their constituent layers by the use of solvents for polyuronides, *Figures 17* and *18*.

As shown by Harlow (13), the residues which remain after treating sections of wood with 72% sulphuric acid and subsequently boiling in 3% sulphuric acid, vary considerably in different species, depending upon the intensity of lignification. In the case of the dicotyledons, the secondary walls of the tracheids and fiber-tracheids frequently dissolve or disintegrate into a finely granular residue, whereas the more intensely lignified walls of the vessels do not, *Figure 14*. In most of the Coniferæ, and in certain of the more heavily lignified dicotyledons, the secondary walls of the tracheary elements swell greatly but do not dissolve or disintegrate. Although the residues of the secondary walls may or may not be present after the action of 72% sulphuric acid, the compact remains of the so-called middle lamella persist. Such "middle lamella" residues always consist of at least three layers, *Figure 21*, and at times may be composed of five layers, *Figure 16*. Five-layered structures occur where the residues of the heavily lignified outer layers of the secondary walls remain closely attached to the residues of the cambial walls and of the intercellular substance. They may be differentiated from the three-layered residues by the structure of the bordered pits (compare (a) in *Figure 16* with *Text figure 2*). If the residues are five-layered, the pits retain their bordered appearance.

Since lignin and pectic compounds are isotropic, the anisotropy of the cambial walls suggests that the cellulose which is present originally persists during tissue differentiation and during the physiological processes of lignification. But what becomes of the polyuronides? Are they replaced by, or transformed into, lignin, or are they merely masked by lignification, which modifies their chemical solubilities, staining reactions, and other properties? Since the so-called middle lamella is intensely lignified, and since the presence of lignin may alter the reactions of the polyuronides, these questions should be attacked by a study of delignified material. It is essential, however, in delignifying wood to use solvents that do not simultaneously remove both lignin and polyuronides. For example, hot sodium sulphite macerates, and removes pectic substances from the cambium and other soft tissues. Thus, the fact that sections of wood are quickly macerated by alternate chlorinations and treatments with hot sodium sulphite does not indicate neces-

sarily that the intercellular layer is composed solely of lignin rather than of a mixture of lignin and polyuronides.

An extensive study of the delignification of wood by standard procedures indicates that delignification and maceration are not necessarily coincident reactions. When sections of various dicotyledons are given alternate treatments with chlorine water and 10% ammonium hydroxide in either aqueous or alcoholic solution at room temperature, until they no longer show traces of the Mäule reaction, *they do not macerate*, although they dissolve completely in 72% sulphuric acid. The delignified sections *may be macerated*, however, by subsequent treatments with standard solvents for polyuronides. In other words, the isotropic intercellular substance of mature wood appears to be composed of two substances, lignin and polyuronides, which may be separated by their differential solubilities.

In delignified sections of wood, the cambial walls react much as they do in freshly cut sections of the cambium. In both types of sections, they persist as a non-cellulose residue after treatment with Schweizer's reagent, *Figure 15*. Furthermore, both types of sections are completely soluble in cuprammonium hydroxide after treatment in solvents of polyuronides. In other words, the cambial walls of the mature wood appear to be composed of a mixture of lignin, cellulose and polyuronides.

If the polyuronides originally present in the cambium persist in the mature xylem, and are more or less masked by lignification, it should be possible to extract substances of a pectic nature from wood. This has been accomplished by Professor Anderson. It should be emphasized in this connection, however, that, if the polyuronides of the so-called middle lamella are of a pectic nature whereas those of the secondary wall are hemicelluloses—as appears to be the case—the yields of the former when computed as percentages of the total dry weight of the wood must of necessity be very low. This is due to the fact that the volume of the cambial walls and intercellular substance is very small in comparison with that of the thick secondary walls. In the cambium and other soft tissues, on the contrary, the intercellular substance and the cambial or primary walls form a high percentage of the total dry weight of the tissue. Therefore, the yields of pectic compounds from a given weight of such tissue is high in comparison with that of mature wood. It is evident accordingly that a transformation of pectic compounds into hemicelluloses and lignin is not essential to account for the fact that soft tissues give high percentages of pectic substances and low yields of hemicelluloses, whereas wood gives high percentages of hemicelluloses and lignin and low yields of pectic compounds.

DISCUSSION

The data recorded in preceding paragraphs indicate that the walls of the cambial initials contain cellulose and are truly anisotropic, and that these walls retain their cellulose and anisotropy during tissue differentiation. Similarly, there is considerable cumulative evidence to indicate that the polyuronides which are present in the walls of the cambial initials and constitute the bulk of the intercellular material, are carried over into the mature wood, and are not entirely replaced by, or transformed into, lignin.

Ritter's (19, 20) arguments for considering that the "middle lamella" is composed largely of "lignin" rather than of pectic compounds are the following: (1) When sections of mature wood are subjected to a standard process for isolating cellulose—i. e., alternate chlorinations and treatments with hot sodium sulphite to remove lignin—the "middle lamella" dissolves, leaving the secondary walls, which are birefringent and are soluble in 72% sulphuric acid. (2) Conversely, when sections are treated with 72% sulphuric acid, the "middle lamella" remains intact, but may be dissolved by subsequent chlorinations and treatments with hot sodium sulphite. (3) When sections are treated with standard solvents of pectic compounds, the "middle lamella" is not dissolved. (4) Ruthenium red is not specific for pectic compounds.

We have shown that delignification and maceration, produced by chlorinations and treatments with dilute alkalis, are not necessarily coincident reactions. Sections of wood may be delignified without dissolving the so-called middle lamella. Such delignified sections may be macerated by the subsequent use of standard solvents for polyuronides. Where sections of wood are macerated by repeated chlorinations and treatments in hot sodium sulphite, the truly isotropic intercellular material dissolves, but the cellulose-containing cambial walls do not. In other words, all of the middle lamella—as defined by Van Iterson, Ritter, and others—does not dissolve when sections are macerated by these standard procedures for removing lignin. Furthermore, we have found that hot sodium sulphite alone is a rapid macerating agent for the cambium and other meristems. Thus, chlorination and hot sodium sulphite remove polyuronides as well as "lignin."

We have shown that the "middle lamella" residues left after treating wood with 72% sulphuric acid always consist of at least three layers and in some cases of five layers. It should be emphasized, in addition, that lignin is not the only substance present in plant tissues that is insoluble in 72% sulphuric acid. Dadswell (8) has shown that much of the "Gerbstoffe" which fills the lumens and saturates the walls of

the xylem cells of *Eucalyptus* and of other genera remains as a residue after standard treatments with 72% sulphuric acid. Our own observations upon sections of a wide range of angiosperms and gymnosperms indicate that, in addition to various types of "Gerbstoffe," coagulated protoplasm and nuclei and the contents of certain types of vacuoles may persist after standard treatments with sulphuric acid. Although the walls of the freshly cut cambium dissolve readily in 72% sulphuric acid, they do not do so after soaking in tannic acid or if they are allowed to absorb certain phenolic substances that are contained in the vacuoles of the living cells. Such facts as these suggest that in the case of wood, the so-called lignin residues may actually consist of a varying mixture of insoluble substances.

The ruthenium red reaction was developed by Mangin (17) during his extensive investigations of the distribution of pectic substances in plant tissues. Although it is not specific for pectic substances, it is extremely useful, when employed with proper precautions, in analytical work. Our investigations of a wide range of organic compounds of known chemical composition indicate that ruthenium red in dilute solutions stains three distinct categories of substances that are of common occurrence in the cambium and its derivative tissues: (1) coagulated protoplasm and nuclei, (2) certain lipoids, and (3) polysaccharides that contain glycuronic or galacturonic acid, e.g., pectic compounds, gums, mucilages, hemicelluloses, oxycellulose, etc. In view of the fact that the first category of substances may be differentiated microscopically and that the second category are removed by preliminary treatments with lipoid solvents, an intense staining of cell walls with ruthenium red is strong presumptive evidence of the presence of polyuronides. Thus, the ruthenium red reaction is extremely useful, not only in studying the distribution of polyuronides in plant tissues, but also in chemical analyses, as a means of visually following the effects of successive steps in the extraction of such substances. We have utilized it effectively in our cooperative investigations with Professor Anderson as a means of modifying chemical techniques for the extraction of pectic compounds and hemicelluloses, and of securing larger yields of these substances from specific tissues.

Ritter's data may be interpreted as demonstrating that the so-called middle lamella is strongly lignified, but they do not provide a reliable basis for concluding that it is composed of lignin rather than of cellulose, polyuronides, and lignin. Our observations, on the contrary, tend to support the views of Schmidt and his co-workers (26, 27), who have argued that lignin occurs in close association with polyuronides.

As stated in our introduction, there is no consensus of opinion concerning the use of such terms as intercellular substance, middle lamella, primary wall, etc. Serious discrepancies in morphological, biophysical, and biochemical investigations have arisen not only owing to differences in definition, but also as a result of inconsistencies in applying specific terms to different tissues and even to the same tissue when different techniques are employed. It is essential to clarify the situation, if possible.

That Dippel and others are not consistent in their use of the terms, *intercellular substance*, *middle lamella*, and *primary wall*, is indicated by a careful study of their texts and particularly of their illustrations of different tissues, *Table 1*. Thus, in dealing with the secondary xylem, Dippel (10) obviously uses the term *middle lamella* in referring to a five-layered structure which consists of two cambial walls, two layers of secondary thickening, and a layer of intercellular material, *Text figure 1*. His isotropic substance—i. e., Van Iterson's (33) "original or true middle lamella" and the middle lamella or primary wall of various other

TABLE I

COMPARATIVE TERMINOLOGIES

Proposed Terminology
for

Soft Tissues		Woody Tissues
	SOFT OR WOODY TISSUES (Text figures 1 and 2)	
Intercellular Substance (Dippel)	= (a) MIDDLE LAMELLA or INTERCELLULAR SUBSTANCE (b)	b+a+b: Intercellular Substance (Dippel); Middle Lamella (Van Iterson and others); Primary Wall (Various botanists)
Primary Wall (Dippel)	= (c) CAMBIAL or PRIMARY WALL	
	(d) OUTER LAYER OF SECONDARY WALL	= Primary Wall (Dippel and others)
	(e) CENTRAL LAYER OF SECONDARY WALL	= Secondary Wall (Dippel and others)
	INNER LAYER OF SECONDARY WALL	= Tertiary Wall (Dippel and others)

c+b+a+b+c = Middle Lamella of Dippel and Intercellular Substance of earlier botanists

writers—actually consists of two feebly anisotropic walls and a layer of truly isotropic intercellular material. His primary wall is the strongly isotropic first-formed layer of secondary thickening. On the contrary, in dealing with the unlignified parenchyma of other tissues, Dippel frequently uses the term *primary wall* in referring to layers that are homologues of the cambial walls, and the term *intercellular substance* in designating the truly isotropic intercellular material. It should be emphasized, in this connection, that many of the most serious discrepancies are due, on the one hand, to confounding the cambial walls with the intercellular material, and, on the other hand, to confusing them with the narrow outer layer of the secondary wall.

The question arises, accordingly, as to what changes are advisable in the definition and use of such terms as *middle lamella, intercellular substance,* and *primary wall.* Are they so firmly established in the literature that they should be retained and redefined, or has their varied use led to so much confusion that they should be replaced by new terms?

Our own conclusions, based upon a detailed study of the cambium and its derivatives and upon preliminary investigations of other meristems and their derivatives, are (1) that the term *primary wall* should not be applied to the first-formed layer of secondary thickening but should be used solely in designating the cambial wall and its homologues in other tissues, and (2) that the term *middle lamella* should be used synonymously with *intercellular substance* in referring to the truly isotropic layer of intercellular material. If some term of convenience is required in referring to those complexes of lignified layers which appear more or less homogeneous under certain specific conditions, Van Iterson's term, *compound middle lamella* is available. It should be clearly recognized, in this connection, however, that the compound middle lamella will be five-layered or three-layered, depending upon the specific techniques used in examining the tissue.

Our reasons for advocating these changes in terminology are the following: There are two distinct and fundamentally different categories of cell walls. Meristematic elements and such of their derivatives as retain a potentiality for growth and enlargement have walls which are characterized by their capacity for growth and extension and for undergoing reversible changes, e. g., in thickness. On the contrary, tissue cells which undergo irreversible changes and thus lose their potentiality for growth and enlargement may form a supplementary or *secondary wall* which tends to be more or less conspicuously laminated.[1] The

[1]This differentiation of primary and secondary walls is similar to that used by Balls (6) in his study of the structure of the cotton hair.

term *primary* wall has been used in referring to the walls of meristematic elements, to the walls of their enlarging derivatives, and to the first-formed layer of true secondary thickening in lignified tissues. To continue to apply the term *primary wall* to two entirely different structures must inevitably perpetuate the existing confusion. Therefore, in view of the fact that the cambial wall—and its homologues in other tissues—is a discrete morphological structure which maintains its identity under all conditions of growth and development, it seems advisable to designate it as the *primary wall*. Any terminology which attempts to differentiate successively formed layers of secondary thickening as primary, secondary, and tertiary walls breaks down completely when applied to all types of cells, i. e., to vessel, fibers, sclerenchyma, etc.

The term *middle lamella*, which was used originally as a substitute for *intercellular substance*, has been employed in the case of lignified tissues in referring to five-layered or three-layered structures and in the case of soft unlignified tissues in designating a layer of truly isotropic material. In view of the fact that there has been an increasing tendency to apply the term to a supposedly isotropic layer in lignified tissues, it seems desirable to restrict the term to the truly isotropic *intercellular substance* which separates the primary walls of adjoining cells.

The objection may be raised that the partition membrane is secreted between the halves of the split cell plate, that it originates as a single unit which has a "primary cleavage plane," and therefore that the two adjacent cambial walls and the subsequently formed intercellular material should be designated as the middle lamella or primary wall. The conception of a cell plate which originates from central thickening of spindle fibers and divides to form two protoplasmic membranes, and of a partition wall which is secreted between the halves of the split cell plate and possesses a predetermined plane of cleavage, rests upon entirely inadequate cytological evidence. It has been severely criticized in recent years by Robyns (21) and others and is questioned by most of those who have worked with living cells and are familiar with the physico-chemical properties of living protoplasm. The colloidal properties of the spindle, the cell plate, and first-formed partition membrane are such that they tend to become more or less profoundly modified during fixation and in injured or plasmolyzed living cells. There is no reliable evidence at present to refute Mangin's suggestion that pectic substances are secreted between the newly formed protoplasmic membranes and that mixtures of cellulose and pectic substances are not formed until subsequently.

In the case of cambial initials and of many other cells, a fraction only of the cambial wall originates during cytokinesis. A large proportion of the wall is formed by the growth and extension of its existing surfaces.

Therefore, in view of the fact that each cambial wall is a discrete morphological structure which maintains its identity under all conditions of growth and development, we do not believe that terminology should be governed solely by phenomena which occur during cytokinesis.

SUMMARY AND CONCLUSIONS

1. A detailed study of the cambium indicates that each initial is enclosed within a wall of its own which is separated from the walls of adjoining initials by more or less intercellular material.

2. The cambial wall is composed largely of cellulose and polyuronides, and is truly anisotropic. It is characterized by its capacity for growth and extension and for undergoing reversible changes in thickness. It is also characterized by possessing plasmodesmata which may be uniformly distributed or aggregated in more or less conspicuous primary pit-fields.

3. The amorphous intercellular material, on the contrary, is composed largely, if not entirely, of polyuronides and is truly isotropic. It is characterized by its plasticity which facilitates those movements and adjustments of cells which are such typical features of the actively growing cambium.

4. In other words, the wall of the cambial initial is a discrete morphological structure which maintains its identity under all conditions of growth and development, whereas the intercellular material is passively molded into various forms and possesses few of the attributes of a true membrane.

5. In the case of those derivatives of the cambium which retain their capacity for growth and enlargement and for undergoing reversible changes, the cambial walls are but slightly modified during tissue differentiation, and no supplementary walls are formed.

6. On the contrary, in the case of tissue cells which undergo irreversible changes and form layers of true secondary thickening, the cambial walls and the intercellular layer become thinner and considerably modified in form during the process of cell enlargement. Furthermore, their optical properties, chemical solubilities, and staining reactions are altered or masked by intense lignification.

7. It is possible to demonstrate, however, that the cambial walls retain their anisotropy during and after tissue differentiation, and, by accurately controlled delignification, to unmask the original chemical solubilities and staining reactions of both the cambial walls and the intercellular material. There is, in fact, much cumulative evidence to indicate that the original cellulose and polyuronides are not completely replaced by or transformed into lignin during tissue differentiation.

8. Thus, the putative "isotropic middle lamella" of the mature xylem is not a homogeneous layer, but consists of two lignified anisotropic cambial walls and an intervening, truly isotropic layer of lignified material.

9. Residues of the so-called middle lamella, obtained by the action of 72% sulphuric acid on mature wood, always consists of at least three layers, i. e. the residues of two cambial walls and of the intercellular material.

10. In macerations produced by repeated chlorinations and treatments with hot sodium sulphite, which dissolves both lignin and pectic compounds, a portion only of the so-called middle lamella dissolves, i. e. the truly isotropic intercellular material. The cellulose-containing cambial walls persist and adhere to the layers of secondary thickening.

11. Delignification of the so-called middle lamella and maceration are not necessarily coincident reactions. By carefully controlled chlorinations and treatments with 10% ammonium hydroxide at room temperatures, sections of wood may be delignified without dissolving the so-called middle lamella. Such delignified sections may be macerated, however, by subsequent treatments with standard solvents of pectic substances. In other words, the isotropic intercellular substance of mature wood appears to be composed of two substances, lignin and pectic compounds, which may be separated by their differential solubilities.

12. Serious discrepancies in the use of such terms as *intercellular layer, middle lamella,* and *primary wall* are due not only to differences in the definition of these terms, but also to inconsistencies in applying them to different tissues, and even to the same tissue when different techniques are employed.

13. As a result of our detailed study of the cambium and its derivatives and of our preliminary investigations of other meristems and their derivatives, we suggest (1) that if the term *middle lamella* is to be retained, it should be used synonymously with *intercellular substance* in referring to the truly isotropic layer of intercellular material and (2) that the term *primary wall* should no longer be applied to the first-formed layer of secondary thickening, but should be used solely in designating the cambial wall and its homologues in other tissues.

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DESCRIPTION OF PLATES

PLATE 110

PHOTOMICROGRAPHS MADE WITH POLARIZED LIGHT AND CROSSED NICOLS. Figs. 3-7 from sections 5 μ in thickness.

Fig. 1. *Pinus Strobus.* Transverse section of the actively growing cambium and its differentiating and fully differentiated derivatives, showing anisotropy of the cambial walls. × 265.

Fig. 2. *Fraxinus americana.* Tangential longitudinal section of the cambium, showing anisotropy of the walls of both ray initials and fusiform initials. × 210.

Fig. 3. *Pinus Strobus.* Tangential longitudinal section of the cambium, showing anisotropic walls of two adjacent fusiform initials and the isotropic intercellular material. The thinner areas of the walls are the so-called primary pit-fields. × 1510.

Fig. 4. *Fraxinus americana.* Tangential longitudinal section of the cambium, showing the anisotropic walls of two adjacent fusiform initials and beaded appearance due to closely approximated primary pit-fields. × 1510.

Fig. 5. *Trochodendron aralioides.* Transverse section of the latewood, showing one entire tracheid and portions of seven adjoining ones. The thick secondary walls are composed of three layers: a narrow, brilliant, outer layer, a brilliant, narrow, inner layer, and a wide intervening dark layer. The brilliant outer layers of adjoining secondary walls are separated by a narrow dark layer, i. e., the so-called middle lamella, which actually consists of two feebly anisotropic cambial walls and a truly isotropic layer of intercellular material (Compare *Text fig. 1*). × 1415.

Fig. 6. *Trochodendron aralioides.* Transverse section of the earlywood, showing one entire tracheid and portions of seven others. Details of structure as in *Fig. 5*, except for the difference in thickness of the central layer of the secondary wall. × 1415.

Fig. 7. *Myodocarpus simplicifolius.* Transverse section of the xylem, showing one entire fiber-tracheid and portions of six adjoining ones. The feebly anisotropic cambial walls are visible between the adjacent outer brilliant layers of the secondary walls. × 1510.

PLATE 111

Fig. 8. *Sequoia sempervirens.* Transverse section of the xylem, unstained and photographed in white light. The inner and outer layers of the secondary wall are more refractive than the wide central layer. The outer layers of adjacent secondary walls blend with the intensely lignified cambial walls and intercellular substance, forming an apparently homogeneous middle lamella which actually consists of five layers. Section of bordered pit at the right. ×1320.

Fig. 9. *The same.* Photographed in polarized light with crossed nicols. In a section of this thickness, 15 μ, the isotropic intercellular substance and feebly anisotropic cambial walls are completely fogged or obscured by the brilliant outer layers of the secondary walls. (Compare *Fig. 5* for section 5 μ in thickness.) × 1320.

Fig. 10. *Fraxinus americana.* Tangential longitudinal section of the phloem, showing but slightly modified cambial walls. Compare *Fig. 11.*) × 800.

Fig. 11. *Fraxinus americana.* Tangential longitudinal section of the cambium, showing walls of ray initials and fusiform initials. × 800.

Fig. 12. *Fraxinus americana.* Tangential longitudinal section of the xylem after standard treatment with 72% sulphuric acid, showing residue of cambial walls which have not thinned down excessively during tissue differentiation. (Compare *Fig. 11.*) × 800.

Fig. 13. *Fraxinus mandshurica.* Tangential longitudinal section of the xylem, showing thick cambial walls between the secondary walls of parenchymatous elements. (Compare *Figs. 11* and *12.*) × 800.

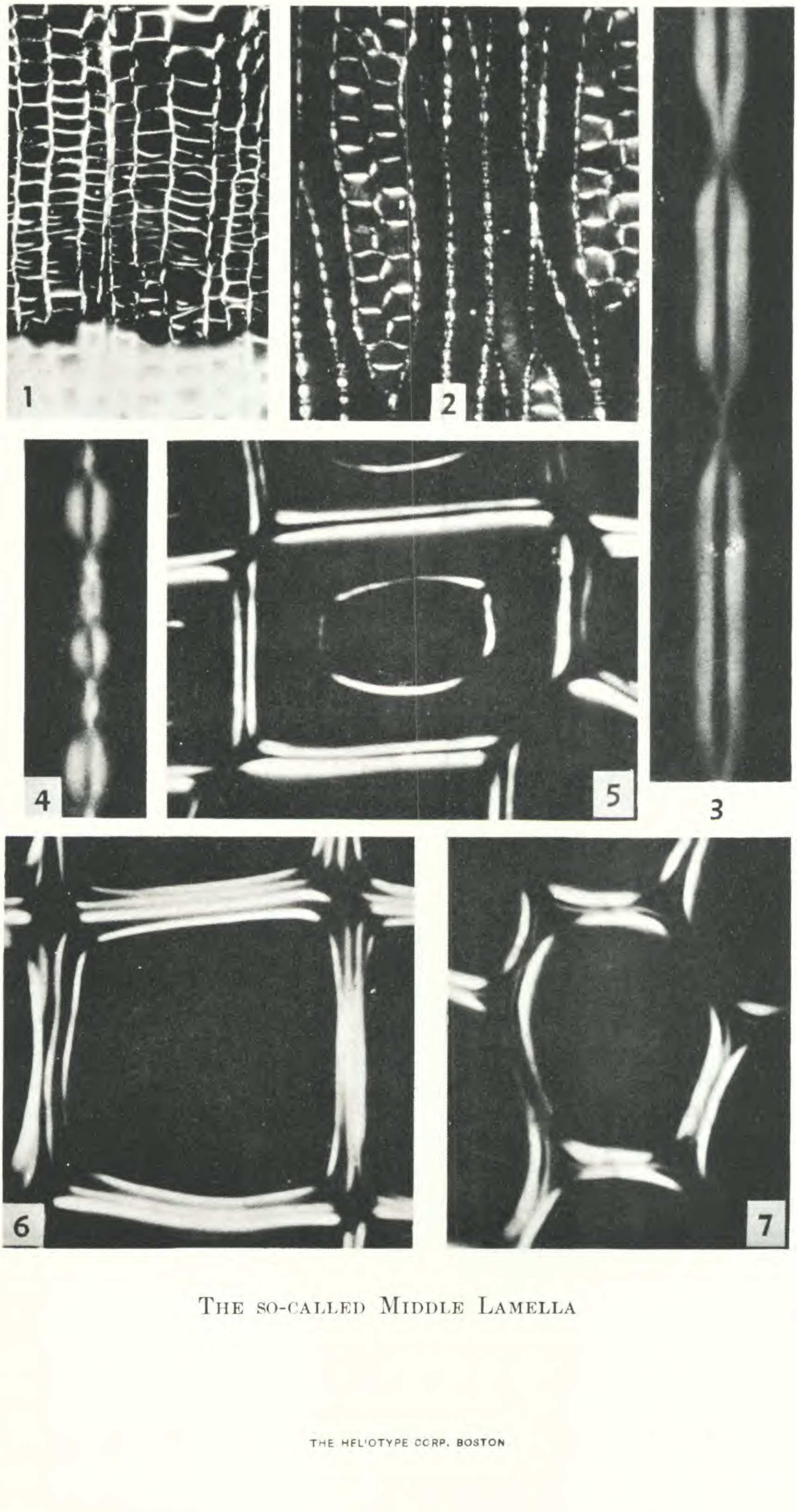
PLATE 112

Fig. 14. *Betula papyrifera.* Transverse section of the xylem after treatment with 72% sulphuric acid. The secondary walls of the fibers have dissolved, whereas the more intensely lignified secondary walls of the vessels have not. × 180.

Fig. 15. *Taxodium distichum.* Transverse section of the xylem after five successive treatments with chlorine water and 10% ammonium hydroxide and extraction with Schweizer's reagent. The intercellular substance and secondary walls have dissolved, leaving the cambial walls only. × 875.

Fig. 16. *Pinus radiata.* Transverse section of the xylem after standard treatment with 72% sulphuric acid, showing five-layered "middle lamella" residue. The five-layered character of the residue may be accurately determined by the structure of the bordered pit at (a). × 945.

Fig. 17. *Sequoia sempervirens.* Tangential longitudinal section of the xylem, showing bordered pit in sectional view. The torus consists of two thickened areas of the cambial walls and an intervening layer of intercellular material. (Compare *Text fig. 2.*) × 2740.



THE SO-CALLED MIDDLE LAMELLA

THE HELIOTYPE CORP. BOSTON